

Inhibition of Reverse Transcriptase Activity Increases Stability of the HIV-1 Core

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Previous studies showed that HIV-1 reverse transcription occurs during or before uncoating, linking mechanistically reverse transcription with uncoating. Here we show that inhibition of reverse transcriptase (RT) during HIV-1 infection by pharmacologic or genetic means increased the stability of the HIV-1 core during infection. Interestingly, HIV-1 particles with increased core stability were resistant to the core-destabilizing effects of rhesus TRIM5 α (TRIM5 α_{rh}). Collectively, this work implies that the surface of the HIV-1 core is dynamic and changes upon the ongoing processes within the core.

Uncoating is the shedding of monomeric capsids from the retroviral core. Since only ~40% of the total capsid in the virion comprises retroviral core (1, 2), a simplified view of uncoating is that the monomeric capsid is in dynamic equilibrium with the assembled capsid (viral core). This implies that the core may exist in a metastable state only in the presence of a high concentration of soluble capsid, keeping the equilibrium shifted toward core formation by mass action; however, the fact that complexes containing capsid have been detected in the cytoplasm implies that cellular factors may be involved in stabilization of the core (3–5). In agreement, elegant experiments have shown that isolated cores undergo reverse transcription more efficiently in the presence of cellular extracts, suggesting the requirement for cellular factors for reverse transcription and uncoating (5–7).

Cumulative evidence suggests that reverse transcription occurs before or during uncoating: (i) TRIM5 α blocks HIV-1 reverse transcription by destabilizing the core (8–10); (ii) HIV-1 cores without Vif, Vpr, or Nef exhibit low stability and a defect in reverse transcription (11); (iii) TRIM5 α mutations that block HIV-1 infection but have lost the ability to block reverse transcription are unable to accelerate uncoating (12); (iv) use of proteasome inhibitors during HIV-1 infection increases the stability of the core and augments reverse transcription (8, 13); and (v) different assays measuring infection of HIV-1 as a surrogate for uncoating suggest that HIV-1 uncoating happens before the completion of reverse transcription (14–16). Overall, this evidence suggests that reverse transcription is mechanistically linked to the uncoating process of HIV-1.

Effect of RT inhibitors on HIV-1 core stability. To test the effect of viral reverse transcriptase (RT) activity on the stability of the HIV-1 core during infection, we used the fate of the capsid (FOC) assay (8–10, 17–19). The FOC assay discriminates pelletable from soluble capsids during infection and allows for the quantification of pelletable capsids or HIV-1 cores, which is a direct measure of core stability. Cf2Th cells containing the empty vector LPCX were first challenged with increasing amounts of HIV-1 green fluorescent protein-expressing (GFP-reporter) virus in the presence of azidothymidine (AZT), and the degree of infection was assessed by measuring the percentage of GFP-positive cells by flow cytometry. As previously shown (20), AZT potently blocked HIV-1 infectivity compared to controls treated with dimethyl sulfoxide (DMSO) (Fig. 1A). We then examined the effect of AZT on stability of the HIV-1 core. For this purpose, we in-

fecting LPCX-transduced cells with HIV-1 in the presence of AZT for 16 h and performed a FOC assay on the infected cells (Fig. 1B). Interestingly, the amount of pelletable HIV-1 capsid increased 3-fold by the use of an RT inhibitor compared to DMSO-treated cells (Fig. 1B and C). As a positive control, we challenged Cf2Th cells stably expressing rhesus TRIM5 α (TRIM5 α_{rh}) (Fig. 1D) with HIV-1 GFP-reporter for the same length of time. TRIM5 α_{rh} destabilizes the HIV-1 core by reducing the amount of pelletable capsids during infection (8, 10). As expected, TRIM5 α_{rh} severely decreased the amount of HIV-1 pelletable capsids during infection (Fig. 1B and C). Overall these results suggested that inhibition of RT by AZT increases the amount of pelletable HIV-1 capsids during infection.

Effects of RT mutants on HIV-1 core stability. Because AZT increases core stability during infection, we hypothesized that HIV-1 viruses bearing mutations in the active site of RT exhibit more stable cores. To test this hypothesis, we used the FOC assay to examine the stability of the HIV-1 core with a D185N mutation in the RT active site. As expected, this mutation allows production of particles that are not infectious (Fig. 2A) (21); however, HIV-1 D185N particles were mature, as determined by Gag processing (Fig. 2B) (22). Subsequently, the effect of RT mutations on the stability of the HIV-1 core was analyzed by the FOC assay (Fig. 2C and D). For this purpose, Cf2Th cells containing the empty vector LPCX were challenged for 16 h with similar amounts of wild-type and mutant viruses normalized by p24 enzyme-linked immunosorbent assay (ELISA). Analysis of soluble and pelletable capsids by the FOC assay revealed that HIV-1 D185N was more stable than wild-type HIV-1 (Fig. 2C and D). As a control, TRIM5 α_{rh} destabilized the HIV-1 core during infection (Fig. 2C and D). These results demonstrated that inhibition of reverse transcription resulted in an increase of pelletable HIV-1 capsid during infection.

Ability of TRIM5 α to destabilize an HIV-1 core bearing a mutated RT. The ability of TRIM5 α_{rh} to restrict infection correlates

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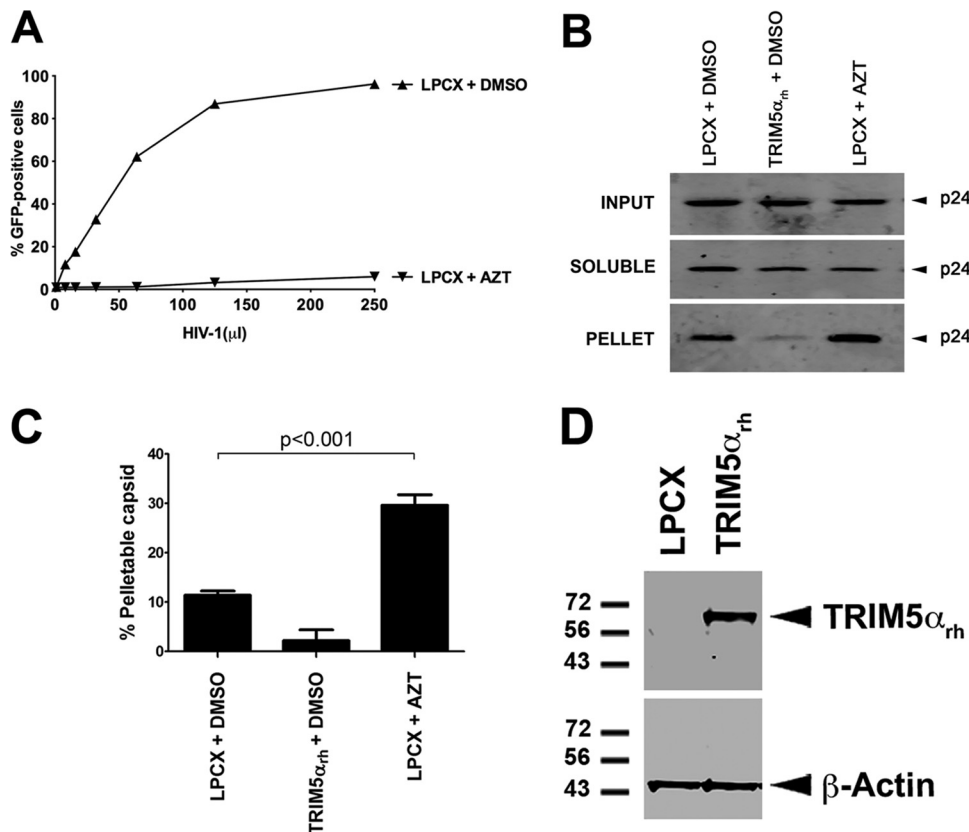


FIG 1 Effects of reverse transcriptase inhibitors on HIV-1 core stability. (A) Cf2Th cells transduced with the empty vector LPCX were challenged with increasing amounts of HIV-1 GFP-reporter virus (800 pg/ml of p24) in the presence of AZT. As a control, LPCX-transduced cells were challenged in the presence of DMSO, the solvent used to resuspend the RT inhibitors. GFP-positive cells were quantified by flow cytometry. (B) LPCX-transduced Cf2Th cells in the presence of AZT or DMSO were challenged with similar amounts of HIV-1 GFP-reporter, and the amount of soluble versus particulate capsid was determined by the FOC assay. As control, we used the same amount of HIV-1 GFP-reporter to perform the FOC assay in Cf2Th cells stably expressing TRIM5 α_{rh} , which is expressed from the LPCX vector. Briefly, cells were incubated with HIV-1 GFP-reporter at 4°C for 30 min, washed, and returned to 37°C. Infection was allowed to proceed for 16 h. Cell extracts were fractionated on a sucrose cushion. Input, soluble, and pellet fractions were analyzed by Western blotting using antibodies against the HIV-1 p24 capsid protein. (C) The percentage of pelletable HIV-1 capsid was determined with respect to the amount of total input capsid. Similar results were obtained in three independent experiments, and standard deviations are shown. Statistical differences are given as $P < 0.001$ (two-way analysis of variance [ANOVA] followed by the Bonferroni posttest). (D) Cf2Th cells stably transduced with TRIM5 α_{rh} and selected in puromycin were analyzed for TRIM5 α_{rh} -hemagglutinin (HA) expression by Western blotting using anti-HA antibodies. The loading control was performed using anti- β -actin antibodies.

with destabilization of the HIV-1 core (8, 10). To test whether the stability gained by the core in our HIV-1 RT D185N mutant could overcome the destabilization induced by TRIM5 α_{rh} , we challenged cells expressing TRIM5 α_{rh} with wild-type and mutant HIV-1 viruses normalized by p24 ELISA. At 16 h postinfection, we separated soluble from pelletable capsids by the FOC assay (Fig. 3A and B). Consistent with our hypothesis, the HIV-1 RT D185N mutant showed an increase of pelletable capsids in the presence of TRIM5 α_{rh} compared to wild-type HIV-1 (Fig. 3A and B). Similarly, we found that AZT and nevirapine inhibit the ability of TRIM5 α_{rh} to decrease the amount of pelletable capsids during infection (Fig. 4A and B). Altogether, these experiments suggested that RT activity within the HIV-1 core is required for the ability of TRIM5 α_{rh} to accelerate uncoating.

These results showed that mutations or drugs that disrupted RT activity increased the amount of particulate HIV-1 capsid by 2- to 3-fold, which is in agreement with previous findings (12, 14–16). An attractive hypothesis that arises from these findings is that the enzymatic activity of RT, which involves conformational changes during synthesis of viral DNA (6), induces the reorgani-

zation of core components in some way that facilitates shedding of monomeric capsids. This effect could be achieved by triggering dissociation of putative stabilizing factors from the surface of the core. In addition, the DNA is a less flexible molecule than RNA, so it is likely that DNA will require more space inside the core. The synthesis of DNA inside the core will probably require more space and will end up rearranging the entire core structure.

In the first hours of infection, while the reverse transcription complex is forming, the viral core undergoes enlargement (4, 23, 24), which is likely to be due to acquisition of host cell proteins. One possibility is that RT activity facilitates recruitment of factors that can trigger uncoating of the virus (6, 25). This notion is in agreement with recent findings of second-site suppressor mutations that rescue virus infection without restoring capsid stability (3, 26); these mutants might be gaining the ability to better recruit host factors that are required for reverse transcription. In contrast, the absence of RT activity might be preventing the normal initiation of uncoating.

The result that the HIV-1 RT D185N mutant was resistant to the core-destabilizing effects of TRIM5 α_{rh} implies that the RT

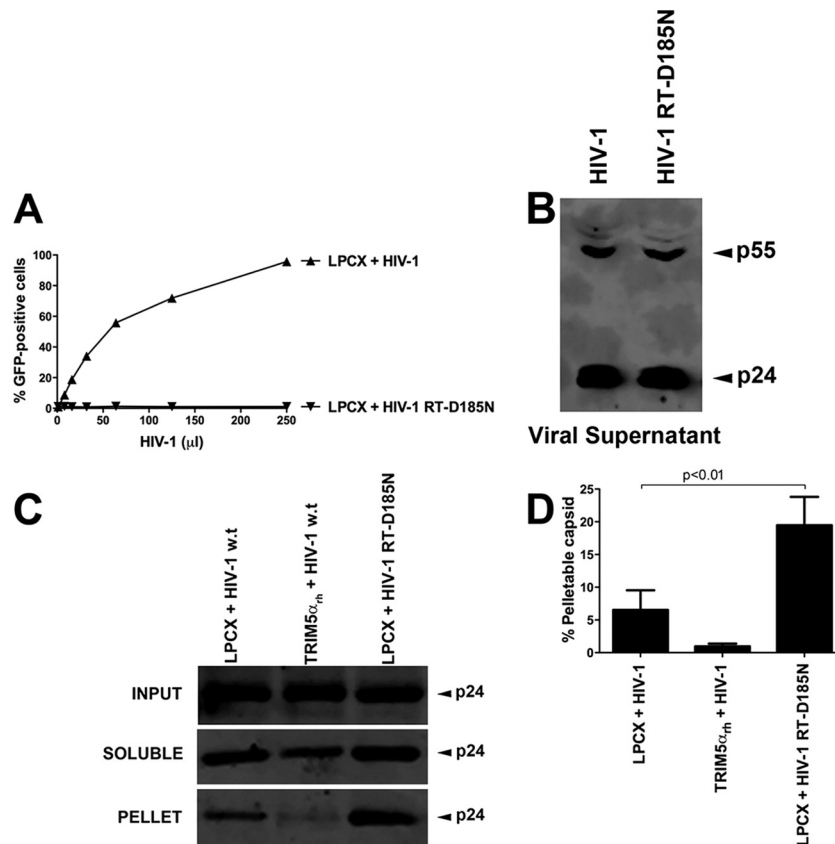


FIG 2 Effects of RT mutants on stability of the HIV-1 core. (A) Cf2Th cells transduced with the empty vector LPCX were challenged with the indicated HIV-1-GFP reporter viruses normalized by ELISA against p24 (800 pg/ml of p24). To study the effect of reverse transcription on core stability, we challenged cells with an HIV-1 strain bearing a mutation in the active site of the RT enzyme (HIV-1 RT D185N). Forty-eight hours postinfection, GFP-positive cells were quantified by flow cytometry. (B) Because the HIV-1 RT D185N mutant is not infectious, Gag-processing levels in viral supernatants were evaluated by Western blotting using antibodies against p24. (C) LPCX-transduced Cf2Th cells were challenged with similar amounts of the indicated HIV-1 GFP-reporter viruses, and the amount of soluble versus particulate capsid was determined by FOC assay. As a control, we used the same amount of wild-type HIV-1 GFP-reporter virus to perform the FOC assay in Cf2Th cells stably expressing TRIM5α_{th}, which is expressed from the LPCX vector. Briefly, cells were incubated with the indicated viruses at 4°C for 30 min, washed, and returned to 37°C. Infection was allowed to proceed for 16 h. Cell extracts were fractionated on a sucrose cushion. Input, soluble, and pellet fractions were analyzed by Western blotting using antibodies against the HIV-1 p24 capsid protein. (D) The percentage of pelletable HIV-1 capsid was determined with respect to the amount of total input capsid. Similar results were obtained in three independent experiments, and standard deviations are shown. Statistical differences are given as $P < 0.01$ (two-way ANOVA followed by the Bonferroni posttest).

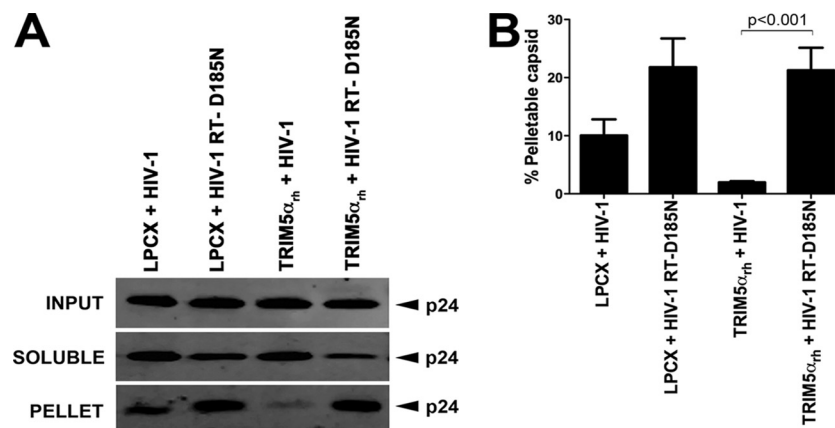


FIG 3 The core-stabilizing HIV-1 RT mutant is resistant to the destabilizing effects of TRIM5α_{th}. (A) Cf2Th cells transduced with the empty vector LPCX or expressing TRIM5α_{th} were challenged with the indicated HIV-1 GFP-reporter viruses normalized by ELISA against p24. The amount of soluble versus particulate capsid was determined by FOC assay. Briefly, cells were incubated with the indicated viruses at 4°C for 30 min, washed, and returned to 37°C. Infection was allowed to proceed for 16 h. Cell extracts were fractionated on a sucrose cushion. Input, soluble, and pellet fractions were analyzed by Western blotting using antibodies against HIV-1 p24 capsid protein. (B) The percentage of pelletable HIV-1 capsid was determined with respect to input capsid. Similar results were obtained in three independent experiments, and standard deviations are shown. Statistical differences are given as $P < 0.001$ (two-way ANOVA followed by the Bonferroni posttest).

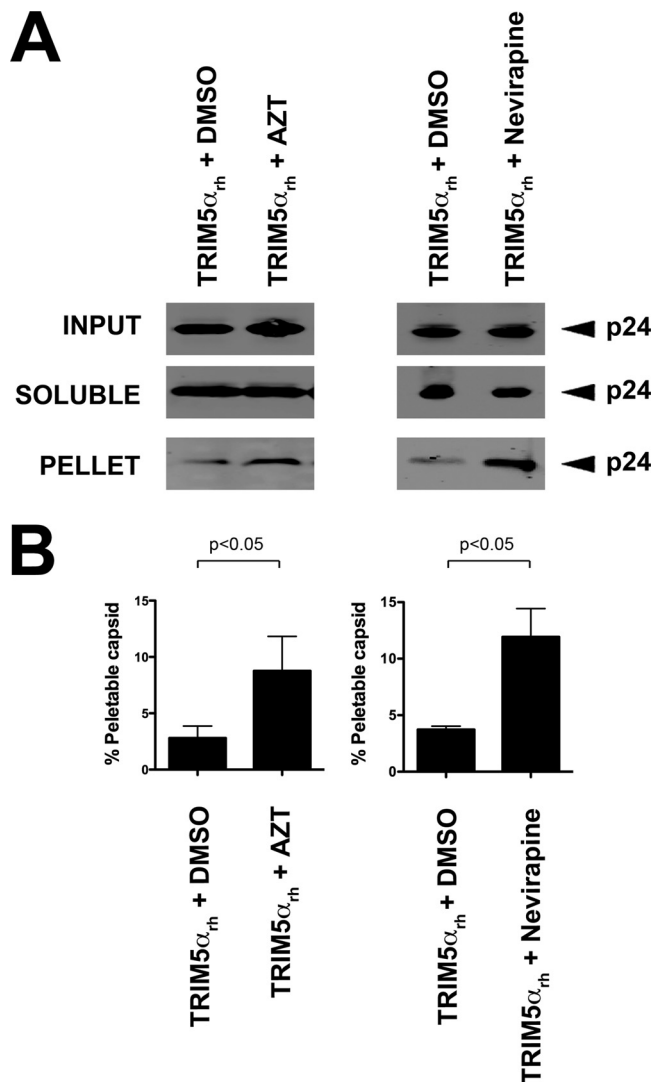


FIG 4 The use of AZT and nevirapine prevents the core-destabilizing effects of TRIM5 α_{rh} . (A) Cf2Th cells stably transduced with TRIM5 α_{rh} were challenged with the indicated HIV-1 GFP-reporter viruses, normalized by ELISA against p24, in the presence of AZT and nevirapine. The amount of soluble versus particulate capsid was determined by FOC assay. Briefly, cells were incubated with the indicated viruses at 4°C for 30 min, washed, and returned to 37°C. Infection was allowed to proceed for 16 h. Cell extracts were fractionated on a sucrose cushion. Input, soluble, and pellet fractions were analyzed by Western blotting using antibodies against HIV-1 p24 capsid protein. (B) The percentage of pelletable HIV-1 capsid was determined with respect to input capsid. Similar results were obtained in three independent experiments, and standard deviations are shown. Statistical differences are given as $P < 0.05$ (two-way ANOVA followed by the Bonferroni posttest).

activity has a role in the ability of TRIM5 α_{rh} to destabilize the core. As suggested above, RT activity within the core might be triggering a conformational change on the surface of the core that allows binding and destabilization by TRIM5 α_{rh} . Previous experiments have demonstrated that an RT-defective HIV-1 abrogates the restriction imposed by TRIM5 α_{rh} to a similar degree as wild-type virus (27); this suggested that the core from the RT-defective HIV-1 particle is still interacting with TRIM5 α_{rh} . A feasible hypothesis is that RT activity triggers dissociation of core-stabilizing

factors, which makes the HIV-1 core more susceptible to the effects of TRIM5 α_{rh} .

Overall, these findings are in agreement with the hypothesis that the surface of the core is dynamic and could expose and hide protein domains, depending upon changes within or outside the core, as shown for other viruses (28, 29). The process of exposing and hiding domains on the surface of the core creates a communication system between the core and its environment, and this communication could be important for the core to sense the appropriate place and time to undergo uncoating.

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REFERENCES

- Briggs JA, Simon MN, Gross I, Krausslich HG, Fuller SD, Vogt VM, Johnson MC. 2004. The stoichiometry of Gag protein in HIV-1. *Nat. Struct. Mol. Biol.* 11:672–675.
- Briggs JA, Wilk T, Welker R, Krausslich HG, Fuller SD. 2003. Structural organization of authentic, mature HIV-1 virions and cores. *EMBO J.* 22:1707–1715.
- Forshey BM, von Schwedler U, Sundquist WI, Aiken C. 2002. Formation of a human immunodeficiency virus type 1 core of optimal stability is crucial for viral replication. *J. Virol.* 76:5667–5677.
- McDonald D, Vodicka MA, Lucero G, Svitkina TM, Borisov GG, Emerman M, Hope TJ. 2002. Visualization of the intracellular behavior of HIV in living cells. *J. Cell Biol.* 159:441–452.
- Warrilow D, Meredith L, Davis A, Burrell C, Li P, Harrich D. 2008. Cell factors stimulate human immunodeficiency virus type 1 reverse transcription in vitro. *J. Virol.* 82:1425–1437.
- Warren K, Warrilow D, Meredith L, Harrich D. 2009. Reverse transcriptase and cellular factors: regulators of HIV-1 reverse transcription. *Viruses* 1:873–894.
- Warrilow D, Warren K, Harrich D. 2010. Strand transfer and elongation of HIV-1 reverse transcription is facilitated by cell factors in vitro. *PLoS One* 5:e13229. doi:10.1371/journal.pone.0013229.
- Diaz-Griffero F, Kar A, Lee M, Stremlau M, Poeschla E, Sodroski J. 2007. Comparative requirements for the restriction of retrovirus infection by TRIM5 α and TRIMCyp. *Virology* 369:400–410.
- Perron MJ, Stremlau M, Lee M, Javanbakht H, Song B, Sodroski J. 2007. The human TRIM5 α restriction factor mediates accelerated uncoating of the N-tropic murine leukemia virus capsid. *J. Virol.* 81:2138–2148.
- Stremlau M, Perron M, Lee M, Li Y, Song B, Javanbakht H, Diaz-Griffero F, Anderson DJ, Sundquist WI, Sodroski J. 2006. Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5 α restriction factor. *Proc. Natl. Acad. Sci. U. S. A.* 103:5514–5519.
- Ohagen A, Gabuzda D. 2000. Role of Vif in stability of the human immunodeficiency virus type 1 core. *J. Virol.* 74:11055–11066.
- Roa A, Hayashi F, Yang Y, Lienlaf M, Zhou J, Shi J, Watanabe S, Kigawa T, Yokoyama S, Aiken C, Diaz-Griffero F. 2011. RING domain mutations uncouple TRIM5 α restriction of HIV-1 from inhibition of reverse transcription and acceleration of uncoating. *J. Virol.* 86:1717–1727.
- Diaz-Griffero F, Perron M, McGee-Estrada K, Hanna R, Maillard PV, Trono D, Sodroski J. 2008. A human TRIM5 α B30.2/SPRY domain mutant gains the ability to restrict and prematurely uncoat B-tropic murine leukemia virus. *Virology* 378:233–242.
- Arfi V, Lienard J, Nguyen XN, Berger G, Rigal D, Darlix JL, Cimarelli A. 2009. Characterization of the behavior of functional viral genomes during the early steps of human immunodeficiency virus type 1 infection. *J. Virol.* 83:7524–7535.
- Berger G, Durand S, Fargier G, Nguyen XN, Cordeil S, Bouaziz S,

- Muriaux D, Darlix JL, Cimarelli A. 2011. APOBEC3A is a specific inhibitor of the early phases of HIV-1 infection in myeloid cells. *PLoS Pathog.* 7:e1002221. doi:10.1371/journal.ppat.1002221.
16. Hulme AE, Perez O, Hope TJ. 2011. Complementary assays reveal a relationship between HIV-1 uncoating and reverse transcription. *Proc. Natl. Acad. Sci. U. S. A.* 108:9975–9980.
17. Berube J, Bouchard A, Berthou L. 2007. Both TRIM5alpha and TRIMCyp have only weak antiviral activity in canine D17 cells. *Retrovirology* 4:68. doi: 10.1186/1742-4690-4-68.
18. Ohkura S, Goldstone DC, Yap MW, Holden-Dye K, Taylor IA, Stoye JP. 2011. Novel escape mutants suggest an extensive TRIM5alpha binding site spanning the entire outer surface of the murine leukemia virus capsid protein. *PLoS Pathog.* 7:e1002011. doi:10.1371/journal.ppat.1002011.
19. Shi J, Zhou J, Shah VB, Aiken C, Whitby K. 2011. Small-molecule inhibition of human immunodeficiency virus type 1 infection by virus capsid destabilization. *J. Virol.* 85:542–549.
20. D'Andrea G, Brisdelli F, Bozzi A. 2008. AZT: an old drug with new perspectives. *Curr. Clin. Pharmacol.* 3:20–37.
21. Mulky A, Sarafianos SG, Arnold E, Wu X, Kappes JC. 2004. Subunit-specific analysis of the human immunodeficiency virus type 1 reverse transcriptase in vivo. *J. Virol.* 78:7089–7096.
22. Bukrinskaya AG. 2004. HIV-1 assembly and maturation. *Arch. Virol.* 149:1067–1082.
23. Fassati A, Goff SP. 2001. Characterization of intracellular reverse transcription complexes of human immunodeficiency virus type 1. *J. Virol.* 75:3626–3635.
24. Warrilow D, Harrich D. 2007. HIV-1 replication from after cell entry to the nuclear periphery. *Curr. HIV Res.* 5:293–299.
25. Warrilow D, Tachedjian G, Harrich D. 2009. Maturation of the HIV reverse transcription complex: putting the jigsaw together. *Rev. Med. Virol.* 19:324–337.
26. Yang R, Shi J, Byeon IJ, Ahn J, Sheehan JH, Meiler J, Gronenborn AM, Aiken C. 2012. Second-site suppressors of HIV-1 capsid mutations: restoration of intracellular activities without correction of intrinsic capsid stability defects. *Retrovirology* 9:30. doi:10.1186/1742-4690-9-30.
27. Besnier C, Takeuchi Y, Towers G. 2002. Restriction of lentivirus in monkeys. *Proc. Natl. Acad. Sci. U. S. A.* 99:11920–11925.
28. Bothner B, Dong XF, Bibbs L, Johnson JE, Siuzdak G. 1998. Evidence of viral capsid dynamics using limited proteolysis and mass spectrometry. *J. Biol. Chem.* 273:673–676.
29. Hilmer JK, Zlotnick A, Bothner B. 2008. Conformational equilibria and rates of localized motion within hepatitis B virus capsids. *J. Mol. Biol.* 375:581–594.